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## Modern Microbiological Investigations in the Central Arctic

By A. E. Kriss

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VESTNIK

In the Spring of this year during the high latitude Arctic Expedition the study of the microbial population of the ocean at the North Pole, undertaken first in 1954, was continued.

New investigations extended the territory of microbiological study to the central Arctic. Personnel who entered in were mycologist M. A. Litvinov, microbiologist O. I. Artmanov, and the author of this paper, with detachments made as if a cross section between the North Pole and North Land. Likewise drifting stations "North Pole-3" (forepart of the termination of the drift) and "North Pole-5" for choice of samples in this region of the Arctic.

Already results of investigations of the past year, partially published, showed in all masses of water of the Arctic Ocean to some thousand meters, and also in the surface layer of mud in the same high latitude, populations of distinct forms of microorganisms. In their vital processes these microorganisms transform organic and inorganic compounds to make conditions favorable for the existence of another form of life—animal and vegetable. It became apparent that in the central Arctic, outside of the immediate mouths of rivers in continental and island run-off and far from the site of the ice melt, there is enriched growth of life, microorganisms long lived in it make the influence biological productivity of polar basins at high latitudes by securing the circulation of organic materials.

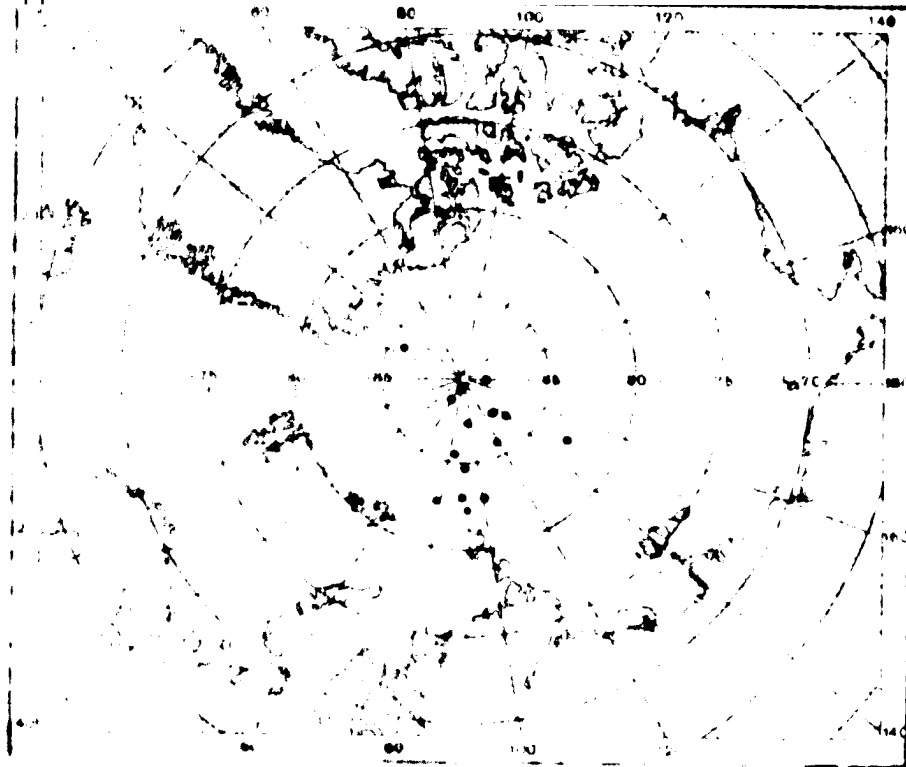
Important microbiological investigations of the ocean, carried out in the North Pole, determine not only that in central Arctic oceanic basins are diverse microbial forms but also reveal their role in the productivity of the water of the polar basin. It was found also that microbiological data may be used in an interesting hydrologic way for indication of the origin and dynamics of water masses. At the junction of water of different densities is a sharp increase in the number of bacteria, seemingly as a result of more intensive propagation of them after calculation with respect to concentration here of organic material.

By the method of calculating cells on ultrafilters after filtering water samples from different ocean depths was established that, for example, in the surface and lower boundaries of penetration in the region of the North Pole Atlantic current the number of microbial populations increases several times in comparison with intermediate and lowest lying levels (Fig. 1).

To show more fully "breaches" in the vertical stratification of microorganisms caused by hydrologic reasons was also investigated with the help of the "glass overgrowing" method.

In the region of the North Pole, glass slides were lowered to depths of 10, 20, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 1000, 1500, 2000, 2500, 3000, and 3500 meters. At these depths they remained 24 hours, and subsequently were withdrawn and after fixing and drying were examined under the high power microscope.

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Disposition of microbiological stations in the central Arctic in 1955. (Marked on map by small solid circles)

In glasses from shallow water 10,000 microorganisms were counted, from depths 10 to 25 meters approximately 10 times less, still deeper only 100 microorganisms were counted. At depths of 200 meters a sharp increase in their number was observed (nearly to 3000) after that once again a decrease to hundreds, ten and at the depth of 1000 meters new significant increases, not different enough in quantity of microorganisms to distinguish it from the surface. Deeper the quantity of bacteria declined to minimum importance (Fig. 2).

The microbiological count method succeeded accurately enough to outline the upper (150 - 200 M) and the lower (about 1000 M) boundaries of the Atlantic current in the region of the North Pole and anew to confirm that at the junction of water masses conditions are created for the growth of bacterial life. This yet more strengthened the necessity for the development of the investigations of microorganisms as hydrologic qualitative indicators.

In the year 1955 fifteen microbiological stations were made. All of them were established in pack ice. Only two of them had to be on the continental slope with depths 400 to 1850 M. The remaining were located in water depth areas of the central polar basin: four stations had depths of 2500 - 2750 M, one station 3000 - 3500 M, and four stations 4200 - 4300 M.

The bathythermographs 1.5 meters in diameter bathometers of the Manson type or a cable were lowered with the help of a special Arctic winch to standard depths at intervals of 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 3000, 4000 meters and deeper, as deep as the cable was used. At certain stations the bathometer was suspended in great depths more often. Besides the water samples taken at these levels, at three stations cores of mud were taken with the help of specially modified Ekman tubes.

On recovery of the bathometer from the proper level, the stopcock was at once carefully closed with a blow torch, and after washing and cooling, certain samples of water were drained. After this the stream of water was directed into sterile 100 ml flasks and into test tubes, in which first was poured 1 ml of ultrafiltered formalin. Samples poured in the flasks were immediately passed without delay across filter membranes No. 2, quantities of 25, 30, 50 ml across each filter (for these tasks microbiological laboratories were arranged in tents on the ice). The filters with the microbic cell sediment from given samples on their surfaces were plated back on meat peptone agar (prepared from sea water taken in the region of the North Pole) and accordingly on meat agar in Petri dishes. In this way growing microbic cells were produced for the study of technical aspects of colonies of microorganisms, including anaerobic fungi which were able to propagate in the laboratory habitat.

Unfiltered samples of water were filtered in 15 ml quantities over bacteria free ultrafilter membranes, prepared by the method of E. A. Buzina and L. I. Gerasimova, for subsequent direct microscopic examination of this water.

Application of the described methods allows for study of the quantity and appearance of the cells of heterotrophic microorganisms, and also of the general number of microbic cells, their morphologic variety and biomass of microorganisms in designated levels of the Arctic Ocean in research in the locality of the central Arctic.

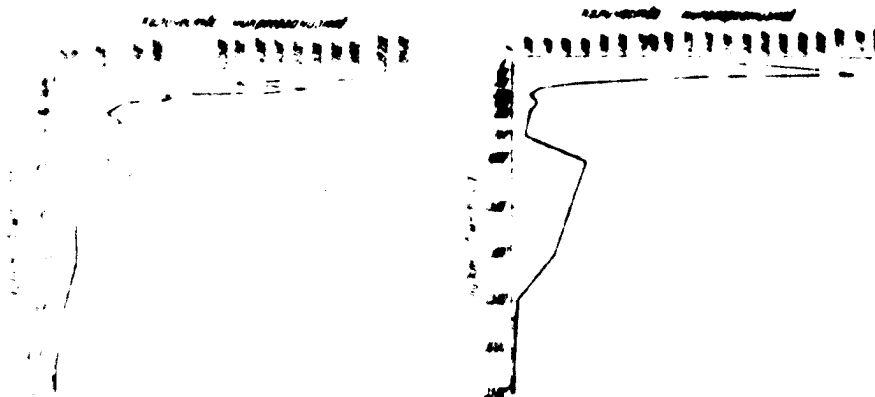


Fig. 1. Vertical distribution of the number of microorganisms in the ocean in the territory of the North Pole from data calculated in filters: to the left in July and to the right in September.

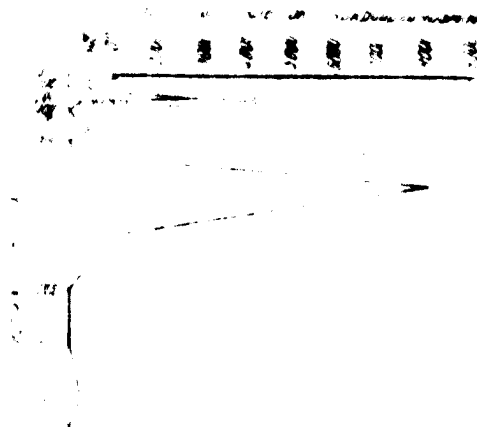


Fig. 2. Vertical distribution of the number of microorganisms in the ocean in the territory of the North Pole from glass overgrowing data.

The surface layer of mud was studied by the method of cultivation. Special tests were set for detecting microorganisms in the mud, not always able to survive, but able to propagate in conditions of a few hundred atmospheres hydrostatic pressure.

In drifting station (North Pole-5) owing to the care and attention of the chief of the station, M. A. Volkova and his assistant, substitute hydrologist Z. M. Godkovich, succeeded in carrying out, besides enumeration research, experiments with glass overgrowing for distinct rates of multiplication of microorganisms in depths of the ocean. With the assistance of rubber holders, glasses were fastened to a cable and lowered to these new standard depths, where water samples were taken with the bathometer. Slides were located at these depths in the current for 2.5 days, and after this were extracted and dried. At each depth two slides were suspended, one of these was fixed, and the other was used for propagation of microbic cells in different media.

As a result of all this work a large amount of scientific material assembled. After financial processing of it, our presentations of the slides to populations of the central polar basin significantly amplify the role of these populations in the productivity of the water of the Arctic Ocean. Interestingly also, owing to the use of a variety of methods and special direct methods in microbiological research, to use microbiological data for characteristics of hydrological conditions in the exploration of the region in the future, it is now feasible.

# Microbiological Research in the Central Arctic in 1956

By A. E. Krice

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In the years 1954 and 1955 microbiological investigations in the central Arctic encompassed circumpolar regions, and also a section situated between the North Pole and North limit. In 1956 these investigations (I. E. Shkumatkin and S. S. Alymov took part in them) were extended in stations to another side of the pole—between it and the Canadian archipelago and Alaska. Part of these stations were added in the region of the pole comparatively inaccessible (see picture 1 and table 1).

Preparations of materials for investigations of the character of the vertical distribution of the number and biomass of microorganisms, and also of their morphological formation in different levels of the water mass, were conducted at 25 stations, including particularly significant water sampling of the Arctic Ocean: 5 of them were over depths up to 1000 M, 7 over depths from 1500 to 2000 M, and 13 over depths beyond 2000 M, to 3000 M. In such a way the majority of the microbiological stations were located over several depths of the polar basin. On each of these stations in pack ice a hole was made and a bathometer of the method of Alchukov was lowered with the help of a winch of this designer to depths: 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500 M. At certain stations, depending on their depth, samples of water were withdrawn from intermediate and deeper levels: 60, 155, 470, 800, 1515, 1700, 1780, 1890, 1860, 1979, 2300, 2370, 2827, 2926, 3122, 3200, 3624, 3650, 3675, 3707, 3750, 3800 M.

Table 1

Coordinates of Microbiological Stations in the Central Arctic

Station No.	Latitude		Longitude		Station No.	Latitude		Longitude	
	North	Eastern	Western			North	Eastern	Western	
1	88°04'3"	208°44'	151°14'		20	76°29'4"	184°00'	176°00'	
2	89°29'5"	294°17'	65°43'		21	84°34'5"	211°21'	148°39'	
3	85°53'	332°30'	27°30'		22	81°34'8"	214°41'	145°19'	
4	88°44'	49°57'			23	77°23'	204°00'	154°00'	
5	89°26'	70°00'			24	77°54'5"	210°28'	149°32'	
6	88°48'	184°30'	175°30'		25	73°03'	211°12'	148°48'	
7	87°23'	89°51'			26	77°00'5"	220°22'	138°30'	
8	85°19'	82°15'			27	78°19'	221°40'	138°20'	
9	84°40'	89°27'			28	78°45'1"	226°09'	133°51'	
10	83°11'	77°37'			29	78°29'0"	228°31'	131°29'	
11	83°27'	87°00'			30	80°25'0"	226°30'	123°30'	
12	83°17'	100°12'			35	81°13'1"	239°09'	153°51'	
13	82°35'	91°22'			36	82°02'	295°00'	104°00'	
14	87°32'	131°23'			37	83°48'1"	241°30'	118°30'	
15	86°38'	140°50'			39	84°39'	264°25'	95°35'	
16	85°46'	116°58'			40	84°10'	224°00'	136°00'	
17	82°55'	151°32'			41	85°27'4"	227°47'	132°13'	
18	78°49'	158°53'			42	87°28'3"	182°47'54"	177°12'4"	
19	76°22'	160°11'			43	86°26'	79°19'12"		

\* Stations No. 1 & 2 were established in 1954; Nos. 3-17 in 1955; Nos. 18-43 in 1956.

в Москву для подсчета числа осевших на их поверхности микроорганизмов и для изучения морфологии этих микробных форм.

В 1956 г. были организованы также микробиологические лаборатории на дрейфующих станциях «Северный полюс-4» (СП-4) и «Северный полюс-5» (СП-5). Стационарные условия работы на этих станциях открыли возможности для проведения исследований со стеклами.

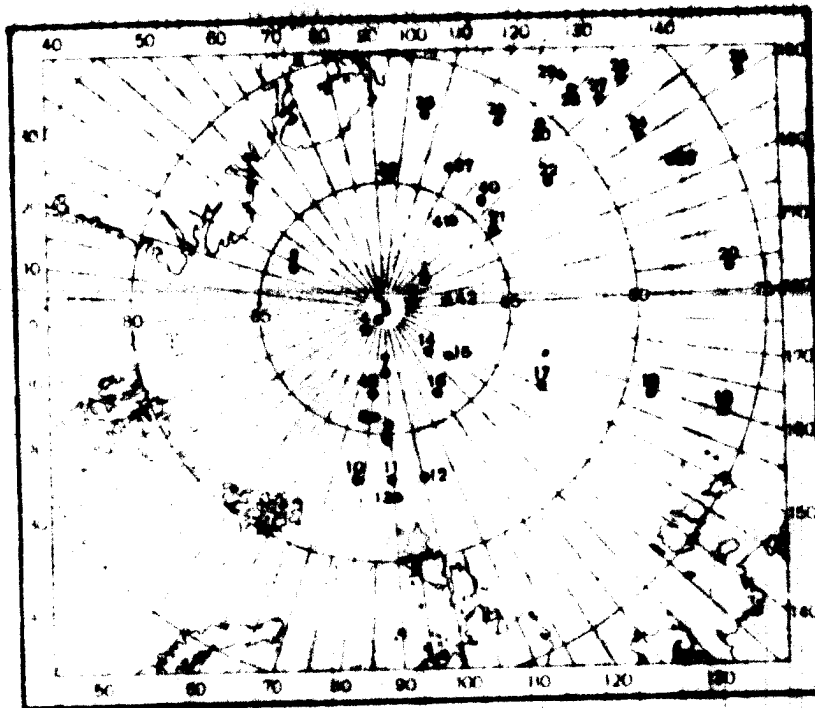


Рис. 1. Карта микробиологических станций в Центральной Арктике.

Стекла, являясь аналогами известных часами, оказались полезными для изучения скорости размножения микроорганизмов в глубинах морей и океанов (2-4). В 1955 г. на дрейфующей станции «Северный полюс-3» (СП-3) стекла были опущены на сутки по стандартным горизонтам до глубины 3300 м. В 1954 г. на дрейфующей станции СП-5 стекла находились на различных глубинах океана до 2500 м 2,5 суток — продолжительность, которой еще не удавалось достигнуть, работая с судна. В 1956 г. на дрейфующей станции СП-4 были проведены опыты с опусков со стеклами.

В резиновых пробках большого формата делались два прореза, в которые вставлялись предметные стекла. Третий прорез служил для укрепления пробки на гидрологическом тросе. С помощью лебедки Алюминиевой-Вики на тросе погружались на горизонты: 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500, 3800 м. Стекла I серии выдерживались на этих глубинах 6 час. Во II серии — 12 час., в III серии — 24 часа, в IV — 7 суток. Извлеченные спустя эти сроки стекла высушивались и фиксировались на пламени спиртовки. В последующее время проводилось микробиологическое изучение осевших и размножившихся на поверхности стекол микроорганизмов.

В микробиологических лабораториях на дрейфующих станциях СП-4 и СП-5 были проведены исследования вертикального распространения гетеротрофных микроорганизмов, способных развиваться на больших средах в лабораторной обстановке. Пробы воды отбирались со стандартных горизонтов батометрии Алексеева, а образцы ила — трубкой Алексеева.

Bathometrically prepared water samples were immediately put into test tubes containing 0.5 ml of ultrafiltered formalin, and filtered in 15 ml quantities across membrane filters free of bacteria. After this the filters were dried and delivered to Moscow for calculating numbers of microorganisms deposited on the surface and for studying the morphology of these microbial forms.

In 1956 microbiological laboratories were organized on drifting stations "North Pole-4" (SP-4) and "North Pole-5" (SP-5). Fixed conditions of work on these stations revealed the possibilities of conducting research with glass. Glasses, making their appearance similarly in suspensions of particles in marine reservoirs, proved useful for study of the rate of reproduction of microorganisms in the deep sea and ocean. In 1955 at drifting station "North Pole-3" (SP-3) glasses were dropped for 24 hours to standard depths up to depth of 3300 M. In 1954 on drifting station SP-5 glasses were located at distinct levels of the ocean to 2500 M for 2.5 days — long duration which failed to come up, working from boats. In 1956 four series of experiments were conducted with glasses on SP-4.

Two notches were made in a large size rubber stopper, in which were inserted glass slides. A third perforation was used fixing the stopper on a hydrologic cable. With the help of an Alekseeva winch, stoppers on the cable were lowered to 5, 10, 25, 50, 70, 100, 150, 200, 250, 300, 400, 500, 750, 1000, 2000, 2500, 3000, 3500, 3900 M. Slides of series I were kept at these levels for 6 hours, series II—12 hours, series III—24 hours, and series IV—7 days. Extracted after these fixed times, slides were dried and fixed in alcohol flame. At present being carried out are microscopic studies of the microorganisms settled and growing on the surface of the slides.

In the microbiological laboratories on drifting stations SP-4 and SP-5 research was conducted on the vertical occurrence of heterotrophic microorganisms, capable of growing in albuminous media in laboratory conditions. Samples of water were obtained from standard levels with Alekseeva bathometer, and mud samples—Alekseeva tube.

After extracting the bathometer from appropriate depths, the stopcock of it was carefully flamed, water samples were drained, and only after this, sterile flasks were filled. 50 ml water samples filtered in Zeitz apparatus across ultrafilter membrane # 2 employed for sanitary microbiological analysis of water. After final filtration, the filters with the microbial cells from a given sample on their surfaces were plated for germination in Petri dishes, rear side on the surface of congealed meat peptone agar (MPA) prepared from sea water, and on Ashby medium (distilled water—1000 ml, invertase—20 g, K<sub>2</sub>PO<sub>4</sub>—0.2 g, MgSO<sub>4</sub>—0.2 g, NaCl—0.2 g, K<sub>2</sub>SO<sub>4</sub>—0.1 g, chalk—2 g). Dishes with filters were put in special metallic baskets, which after this were hung toward the dome of the tent. In the dome of the tent the temperature was 25-27° owing to the heating of the tent gas plate.

After 4-5 days incubation at this temperature some filters developed colonies of microbes. They developed on MPA or Ashby medium and were microscopic.

In table 2 reduced data of quantity of heterotrophic microorganisms (according to number of colonies growing) at different depths in the Arctic Ocean in July and September 1954, and in the Spring of 1955 and 1956. These microorganisms permit the possibility to judge concerning the concentration and distribution of easily utilisable form of organic material, that is, organic material occurring in the elementary phase of decomposition.

Table 2

Quantity of microorganisms utilizing readily assimilable organic material in different ocean depths in central Arctic (number of colonies in 1 liter of water)

Depth in meters	July 1954	September 1954	May 1955	May 1955	April 1956		May 1956	
	SP-3 (88°04' N lat, 151°16' W long)	SP-3 (89°29' N lat, 65°43' W long)	SP-4 (82°55' N lat, 151°32' W long)	SP-3 (85°53' N lat, 27°30' W long)	SP-4 (87°28' N lat, 177°12' W long)	Active	SP-5 (86°06' N lat, 79°29' W long)	Active
0	MPA	MPA	MPA	MPA	MPA		MPA	Active
10	Solid growth	525	120	160	80	120	0	0
20	1120	1995	120	0	0	0	0	0
30	35	2485	0	0	0	0	0	0
40	315	390	0	0	20	0	20	0
50	490	795	0	0	0	0	0	0
60	2160	140	0	0	0	0	40	0
70	200	2660	80	0	0	0	20	0
80	200	3045	0	0	0	0	0	0
90	200	665	0	0	0	0	0	0
100	Solid growth	980	0	0	0	0	0	0
110	200	105	0	0	0	0	0	0
120	200	245	0	0	0	0	0	0
130	200	0	0	0	0	0	0	0
140	430	175	0	0	0	0	0	0
150	175	105	0	0	0	0	0	0
160	200	210	0	0	0	0	0	0
170	200	245	0	0	0	0	0	0
180	200	35	0	0	0	0	0	0
190	0	390	0	0	0	0	0	0
200	0	140	0	0	0	0	0	0

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stations, with the exclusion of one, occurring at about 86° in the pole, the possibility to characterize the circumpolar region in Spring, Summer and Fall periods in different years. By inspection of Table 2 one is struck by clear differences in the contents of heterotrophic microorganisms in the seasonally assimilable organic material in July and September and in the other months. In the Summer and in the beginning of Fall data of the pattern of microorganisms was uncovered in nearly all ocean depths in the vicinity of the North pole. In the Fall large quantities were found in the region of the junction of the Atlantic current with tendency to higher levels of water. This boundary layer one may observe as a layer of temperature discontinuity, with this same difference, that here arises a break in the direction of a rise in temperature--transition from a negative temperature of water toward a positive in the Atlantic layer.

In the Spring of 1955 and 1956 the concentration of heterotrophic microorganisms in alluminous media proved to be in all water strata so little, that on ultrafilter membranes not one colony grew after filtering 50 ml of water across them.

Spring surveys showed that only in certain samples taken in the upper layer of the ocean grow colonies of heterotrophic microorganisms. But in the background of so abrupt an impoverishment of water strata by this sort of microorganism, all now emanate from 150-meter depth, near the upper boundary of the Atlantic current. At nearly all stations in April-May here there was an increase of concentration of heterotrophs in comparison with higher and underlying levels.

Resulting data forces the assumption of the presence of seasons in the development of life in the central part of the Arctic Ocean under pack ice. After summer-fall rise, combined with the action of radiant radiations, with the onset of the dark time of the year, in spite of practically no fluctuation in water temperature, comes a depression of biological productivity of the ocean in the central Arctic. With the completion of the polar night also appears an abrupt decrease in the number of technical kinds of microorganisms which utilise in their activity products of exchange of different hydrobiota and of dead bodies of planktonic organisms which were beginning to decompose.

Concurrently with the study of water samples on drifting station SP-4 and SP-5 were conducted microbiological studies of the top layer of mud, taken with an Alekseeva tube at SP-4 at depth 3950 M, and at SP-5 at depth of 3544. Immediately after extraction, the mud was inoculated into MPA and Ashby medium in 0.1 ml suspension of mud, prepared in dilution 1:10 at SP-4 and dilution 1:25 at SP-5. After incubation at temperature 25-27°, grew colonies from some of the mud samples on MPA--neither from SP-4 nor SP-5. On Ashby medium from mud taken at SP-4 in one replicate 4 colonies grew, in the other, 2 colonies.

Colonies of microorganisms grown from water samples and from mud consisted of nonsporogenous and sporogenous bacilli, spirobacteria and cocci forms. Isolations also of yeast colonies.